Surveillance of Porcine RVC in Commercial Swine Herd from Ohio

Annika Diaz, Juliet Chepngeno, Linda Saif, and Anastasia Vlasova

The Ohio State University
Ohio Agricultural Research and Development Center
Department of Veterinary Preventive Medicine

Abstract

Rotavirus C (RVC) causes severe diarrhea in young piglets, often resulting in death. We aimed to identify the prevalence and quantity of RVC in samples from nursing piglets from a commercial swine herd in Ohio. Rectal swabs from nursing piglets with and without scours were collected. Using RT-qPCR, we demonstrated that 86 out of 113 samples (76.1%) were RVC positive, indicating high RVC prevalence as reported previously. There was a significant positive relationship (p=0.018) between the quantity of RVC RNA detected in piglets with scours compared to healthy piglets. We also noted a significantly higher number (p=0.0009) of litters with scours born to gilts (sows of 1st parity) than to higher parity sows. Thus, our results suggest that piglets born to gilts are at higher risk of developing symptomatic or more severe RVC gastroenteritis. Future studies will determine whether it's associated with insufficient lactogenic protection provided by gilts or other factors.

Introduction

Rotavirus infections are known to cause diarrhea in both pigs and humans. Piglet death as caused by diarrheic diseases is a major problem experienced internationally. Group C rotavirus was first found in pigs in 1980 (Saif et al., 1980), followed by its discovery in cows, humans, ferrets, and dogs (Martella et al., 2007). The virus poses a particularly large issue for the pig industry as young piglets can experience high rates of morbidity after contracting the virus. Clinical infection signs of RVC are more prevalent in younger piglets, while older pigs with the disease are generally asymptomatic (Tuanthap et al., 2018). For many years, only one full genomic sequence of RVC was available called Cowden, which ultimately limited the analysis that could be drawn in regard to the behavior and evolution of the disease. Additionally, for

decades, it remained the only porcine RVC adapted to cell culture growth, which has been a major limiting factor in RVC research.

In a study focused on rotavirus infections, it was found that suckling pigs were more prone to becoming infected with a rotavirus than weaned piglets, as 21.1% of nursing piglets had rotavirus A (RVA) while only 2.1% of weaned piglets had the virus (Amimo et al., 2013a), which was likely due to the fact that the suckling pigs' immune system is immature or the lack of the prior exposure to RVC. Similar trends are present in the human population impacted by RVC, in that children are much more susceptible to infection from the virus, as it is commonly seen in newborns to three-year old's (Gabbay et al., 2008). Both humans and pigs may experience similar symptoms, such as diarrhea and vomiting, which have been shown to be potentially life threatening in both cases. In humans, immunity to rotaviruses appears to be worsened with malnutrition, dehydration, and secondary infections, as is seen with increased rates of child morbidity in developing countries (Parashar et al., 2003). Similar factors may be analyzed in regard to pig morbidity and mortality, especially due to the similarity in symptoms across species. New studies reveal that RVC may also be zoonotic, due to similarities in human and porcine strains as observed in children testing RVC positive (Gabbay et al., 2008).

There is an evident need for a better understanding of RVC epidemiology and pathogenesis due to it being relatively newly discovered and its high and increasing prevalence across a variety of species, including humans. With an increasing presence of RVC infections being seen in both piglets and sows, questions regarding maternal immunity and acquired piglet immunity are of high importance. Extensive research and development of vaccines for the historically prominent RVA have allowed for some control of infection, while RVC has been able to become more

prevalent in piglets in several farms across Ohio (Amimo et al., 2013b). Additionally, there is a limited understanding of how maternal immunity plays a role in piglet's protection against RVC. However, suckling piglets are known to develop RVC diarrhea very often, while RVA diarrhea is uncommon prior to piglet weaning (Marthaler et al., 2014). Thus, it's important to explore prevalence and maternal protection of suckling piglets against RVC, which poses a large threat to the swine industry.

Materials and Methods

1.a. Swine herd sample collection

A swine herd from Ohio, USA was used in surveillance of the RVC virus. Blood and milk samples from 30 sows were collected, of which 15 sows had litters presenting with diarrhea and the remaining 15 sows had healthy litters. Rectal swabs were collected from the 30 sows' suckling piglets (n= 4/litter, 113 total piglets) ranging from 2 to11 days of age and were stored at 4° C until processing (Table 1). The litters were classified as having diarrhea if presenting with liquid or watery feces, or healthy if presenting with solid or paste-like feces.

Maternal parity	Total piglets born	Pig age @ time of sampling	Number of Sampled Piglets	Piglets Diarrhea Status	Sample collection date
1	12	2 Days	4	Diarrheic	May 2018
1	12	3 Days	4	Diarrheic	May 2018
1	14	3 Days	4	Diarrheic	May 2018
1	10	4 Days	4	Diarrheic	May 2018
1	16	4 Days	4	Diarrheic	May 2018
1	16	4 Days	4	Diarrheic	May 2018
1	11	2 Days	4	Diarrheic	May 2018
4	16	5 Days	4	Diarrheic	May 2018
5	16	4 Days	4	Diarrheic	May 2018
1	19	7 Days	4	Diarrheic	May 2018
4	17	5 Days	4	Diarrheic	May 2018
1	14	5 Days	4	Diarrheic	May 2018
1	17	6 Days	4	Diarrheic	May 2018
1	2	5 Days	4	Diarrheic	May 2018
4	16	10 Days	4	Diarrheic	May 2018
3	16	11 Days	4	Healthy	May 2018
4	21	8 Days	4	Healthy	May 2018
4	16	8 Days	4	Healthy	May 2018
4	15	5 Days	4	Healthy	May 2018
2	10	6 Days	4	Healthy	May 2018
5	18	4 Days	4	Healthy	May 2018
4	19	4 Days	4	Healthy	May 2018
4	16	4 Days	4	Healthy	May 2018
4	17	5 Days	4	Healthy	May 2018
3	17	6 Days	4	Healthy	May 2018
5	18	4 Days	4	Healthy	May 2018
5	17	5 Days	4	Healthy	May 2018
5	19	4 Days	4	Healthy	May 2018
2	12	3 Days	4	Healthy	May 2018
4	20	3 Days	4	Healthy	May 2018

Table 1: List of sows sampled from a commercial swine herd, showing parity, number of piglets in the litter, and diarrhea status.

1.b. Swine herd rectal swab sample processing

Rectal swabs were agitated in 2 mL of 1x Minimum Essential Medium (MEM) and 1% antibiotic-antimycotic (Anti-Anti) (Life Technologies, Grand Island, NY, USA) to suspend any present fecal matter. The tubes were centrifuged at 2095 *g* for 20 min at 4° C, followed by extraction of the supernatant. Using the MagMAX total RNA isolation kit (Life Technologies, Grand Island, NY, USA) and the manufacturer's protocol, genomic RNA was extracted (50 μL) from the rectal swab supernatant.

1.c. Swine herd rectal swab qRT-PCR

qRT-PCR was performed for all rectal swab RNA samples using One-step RT-PCR Kit (Qiagen, Germantown, MD) using the primers and probe listed in Table 2 (Marthaler et al., 2014). RT-qPCR was carried out using the following protocol: reverse transcription at 50℃ for 30 min, initial PCR activation at 95℃ for 15 min, 40 amplification cycles with denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, extension at 72℃ for 1 min, and final extension at 72℃ for 10 min. All data were converted to shedding titers via calculation using a standard curve and then converted to FFU/mL. A confirmed RVC-positive sample was used for a positive control and RNA free water was used as a negative control.

Target or function		Sequence	Region (nt)	Reference /source
Detection	RVC	Forward: 5'-ATGTAGCATGATTCACGAATGGG-'3 Reverse: 5'-ACATTTCATCCTCCTGGGGAT-'3 Probe:5'-VIC-GCG TAG GGG CAA ATG CGC ATG A-TAMRA-3'	1252-1339	Marthaler et al, 2014

Table 2: Genome sequence used in the primers and probe for RVC detection in RT-PCR.

1.f. Statistical Analysis

To determine the relationship between the parity of a sow and the occurrence of diarrhea in their litter, a correlation test was used. Significance was determined at $p \leq 0.05$ for all comparisons. All statistical analyses were performed using GraphPad Prism 7.0c (GraphPad Software Inc. CA, USA).

Results

2.a. qRT-PCR of fecal swabs from Ohio swine herd

Of 113 rectal swab samples, 86 samples were determined to be RVC positive, indicating an overall RVC prevalence of 76.1% (diarrheic= 82.5%, healthy = 69.6%) as illustrated in Figure 2.

Diarrheic piglets were determined to have significantly higher (p= 0.0118) RVC shedding titers compared to healthy piglets (Figure 1). Additionally, gilts (first time mothers) were determined to be significantly (P= 0.0009) more likely to have litters with piglets presenting clinical signs of RVC, specifically diarrhea (Figure 3).

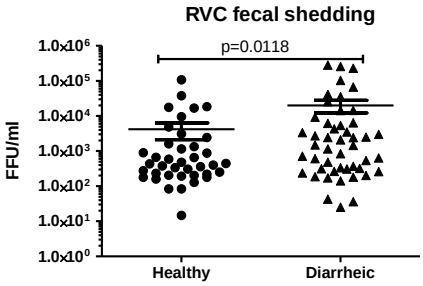

Figure 1: Titers of RVC fecal shedding titers in FFU/mL for healthy and diarrheic piglets. Healthy piglets have a significantly higher (p=0.0118) average RVC shedding titer.

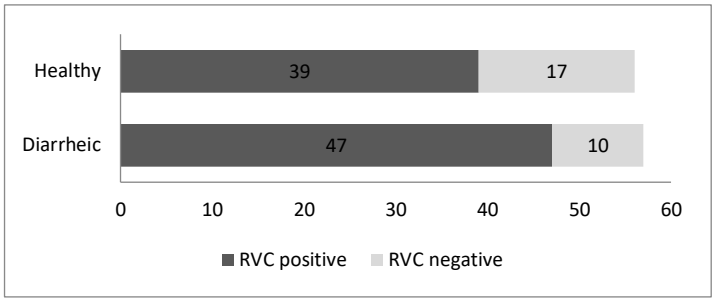

Figure 2: The numbers of healthy and diarrheic piglets that tested RVC positive or negative, showing an overall RVC prevalence of 76.1%. 82.5% of diarrheic piglets and 69.6% of healthy piglets tested RVC positive.

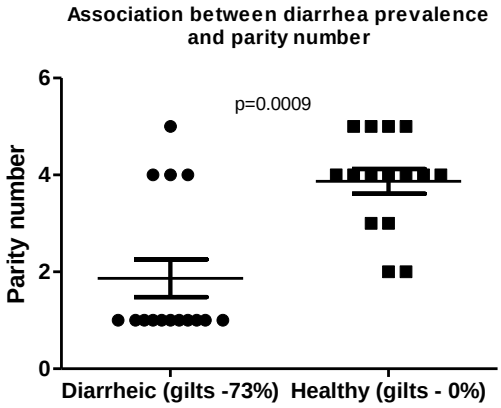

Figure 3: Graph showing the correlation between parity number of sows with diarrhea prevalence in their piglets. First parity sows (gilts) had significantly higher (p=0.0009) prevalence of diarrhea in their piglets.

Discussion

The data from this study show that RVC is afflicting a large percentage of a commercial swine herd, which ultimately leads to economic loss either via euthanasia or alternative treatment

methods. In several studies, it was found that while RVC causes diarrhea, the virus has been found in pigs with and without diarrhea (Theuns et al., 2016), which exemplifies the importance of evaluating piglets that present with diarrhea, as well as those that do not. The data from this study showed that healthy piglets from the Ohio swine herd were also carrying RVC (69.6%). These asymptomatic cases of RVC are not uncommon, as evidenced through a study that gathered samples from pigs that did not show any signs of infection, which focused on analyzing the percent of asymptomatic piglets carrying the virus (Amimo et al., 2017). The results of that study showed that RVC can be present regardless of whether or not a pig is showing signs, an important fact to consider in further research. Despite the prevalence of piglets testing RVC positive without presenting with diarrhea, it is still more commonly seen that diarrheic piglets are generally more likely to have RVC (Amimo et al, 2013b). This trend was also evident in our study with a significantly higher ($p= 0.0118$) average RVC fecal shedding titer for diarrheic piglets, compared to healthy piglets. Additionally, we were able to determine a key correlation between gilts ($P= 0.0009$) having litters presenting with diarrhea, the characteristic clinical sign of RVC infections. Future studies are underway to assess the relationship between parity and the prevalence of RVC, while focusing on determining the role lactogenic maternal immunity plays in those trends.

References

Amimo, J. O., Machuka, E. M., & Okoth, E. (2017). First detection of rotavirus group C in asymptomatic pigs of smallholder farms in East Africa. Pathogens,6(3), 37.

Amimo, J. O., Vlasova, A. N., & Saif, L. J. (2013a). Detection and genetic diversity of porcine group A rotaviruses in historic (2004) and recent (2011 and 2012) swine fecal samples in Ohio: Predominance of the G9P[13] genotype in nursing piglets. Journal of Clinical Microbiology,51(4), 1142-1151.

Amimo, J., Vlasova, A., & Saif, L. (2013b). Prevalence and genetic heterogeneity of porcine group C rotaviruses in nursing and weaned piglets in Ohio, USA and identification of a potential new VP4 genotype. Veterinary Microbiology,164(1-2), 27-38.

Gabbay, Y. B., Borges, A. A., Oliveira, D. S., Linhares, A. C., Mascarenhas, J. D., Barardi, C. R., . . . Jiang, B. (2008). Evidence for zoonotic transmission of group C rotaviruses among children in Belém, Brazil. Journal of Medical Virology,80(9), 1666-1674.

Martella, V., Bányai, K., Lorusso, E., Bellacicco, A. L., Decaro, N., Camero, M., . . . Pezzotti, G. (2007). Prevalence of group C rotaviruses in weaning and post-weaning pigs with enteritis. Veterinary Microbiology,123(1-3), 26-33.

Marthaler, D., Homwong, N., Rossow, K., Culhane, M., Goyal, S., Collins, J., . . . Ciarlet, M. (2014). Rapid detection and high occurrence of porcine rotavirus A, B, and C by RT-qPCR in diagnostic samples. Journal of Virological Mehtods,209, 30-34.

Parashar, U. D., Hummelman, E. G., Bresee, J. S., Miller, M. A., & Glass, R. I. (2003). Global illness and deaths caused by rotavirus disease in children. Emerging Infectious Diseases,9(5), 565-572.

Saif, L.J., Bohl, E.H., Theil, K.W., Cross, R.F., & House, J.A., 1980. Rotavirus-like, calicivirus-like, and 23-nm virus-like particles associated with diarrhea in young pigs. Journal of Clinical Microbiology, 12, 105–111.

Theuns, S., Conceição-Neto, N., Zeller, M., Heylen, E., Roukaerts, I. D., Desmarets, L. M., . . . Matthijnssens, J. (2016). Characterization of a genetically heterogeneous porcine rotavirus C, and other viruses present in the fecal virome of a non-diarrheic Belgian piglet. Infection, Genetics and Evolution,43, 135-145.

Tuanthap, S., Phupolphan, C., Luengyosluechakul, S., Duang-In, A., Theamboonlers, A., Wattanaphansak, S., … Poovorawan, Y. (2018). Porcine rotavirus C in pigs with gastroenteritis on Thai swine farms, 2011-2016. PeerJ, *6*, e4724.